Steven M. Ruben
Appl. No. 10/662,429

Department
Subject
Name ANIL SIM # 6
Address 5/5/94 - [illegible]

National Brand 43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

75 Sheets
9¾" x 9¾"
Quad.

0 73333 43648 8

Ruben EXHIBIT #89

Department

Subject

Name ANN SIM

Address 5/5/94 -

National Brand

43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

7 Sheets
x 9¼"
Quad.

0 73333 43648 8

Pg 138 Book #127
AMIC #5

6/13/94

Inoculate 100 ml TB+Amp
Single colony of HSH120
and HTPAN08
Incubate 37°C w/ Aeration
overnight

6/14/94

TIP - 500 - Maxi Prep.
Spin cells 5K 15 min
Pour off Supernatant
Resuspend pellet in 10ml P1+RNase
Add 10 ml P2
Let sit at RT 5 min
Add Cold P3 - 10 ml
Incubate on ice 20 min.
Spin 9K 30 min 4°C.
~~Transfer Supernatant to fresh~~

Equilibrate TIP-500 with 10ml QBT buffer
Transfer Supernatant to Column -
Strain through Kimwipe
Wash tip 2x 30ml QC Buffer
Elute with 15 ml QF buffer
Add 10.5 ml Isopropanol & mix
Sit on ice 10 min.
Spin 9K 30 min
Pour off Supernatant
Wash pellet 15ml 70% Ethanol
Let pellet Air dry.
Dissolve pellet in 400 µl TE

 Maxi Prep HTPAN08 & HSUSH20

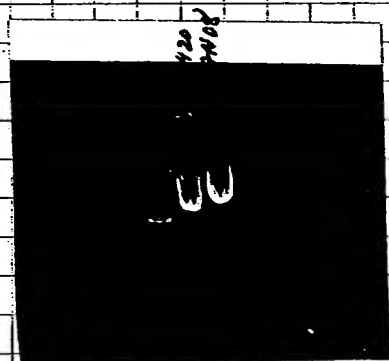
6/14/94

Read OD 260/280

Dilute 1:200

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
1 HSUSH20	0.1716	0.1051	0.0058	1.6688	0.5992	1.7 ug/ul
2 HTPAN08	0.1617	0.0983	0.0060	1.6862	0.5931	1.6 ug/ul

Run 1ul on gel with pBSK.



froze Glycerol Stocks -80°C

Pg 136

6/15/94

~~Want to check if primers made for sequencing are ok. Sequence of HTAS88, HSBAF83 and HSAAG51 ... primers~~ Pg 59

128 HE20I42/HSUSH20 2° Screen

7/20/94

Incubate 3 hrs at 37°C
Heat 70°C 20 min.
Store 4°C.
Use 5ul to transform 200ul SOLR — Plate 50ul.

7/21/94

Plates look Very dense
Do PCR on Rescue.

HSU			HE2	
HSUSH20FP04	0.15		HE20I42FP03	0.15
	0.15	M13R		0.15
	3.2	10xdNTP		3.2
	3.2	10xPCR		3.2
	23.1	H_2O		23.1
	0.2	Taq		0.2
	2	Rescue		2
	32			32

PCR Program #69.

95°C - 5min
95°C 20sec
55°C 20sec } 30x
72°C 1min
72°C 7.5min.
4°C

⊕ Controls plasmid DNA.

Run Well on gel with ⊕ Controls and 1kb ladder.

Steve Rich
7/21/94

136 HEQMF73/HTPAN08 - Protein Exp

7/26/94

PCR - Protein Exp.

(pg 54)

2.5 ng/λ HEQMF73S07 & S05 - NFE2
2.5 ng/λ HTPAN08 S04 - TNFα - Fas.

①		②	
HEQMF73S07	1	HEQMF73S05	1
2245 3' Bgl II Stop	1	3' Bgl II Stop 2245	1
2242 5' Bam HI Start	1	5' Bam HI Start 2242	1
10x dNTP	5	10x dNTP	5
10x PCR	5	10x PCR	5
H_2O	36.8	H_2O	36.8
Taq	0.2	Taq	0.2
	50		50

③		④	
HTPAN08 S04	1	HTPAN08 S04	1
2244 5' Nco Start 251	1	2243 5' Nco Start 185	1
2241 3' Bam HI Stop.	1	2241 3' Bam HI Stop.	1
10x dNTP	5	10x dNTP	5
10x PCR	5	10x PCR	5
H_2O	36.8	H_2O	36.8
Taq	0.2	Taq	0.2
	50		50

⑤		⑥	
HTPAN08 S04	1	HTPAN08 S04	1
2239 5' Bam HI Start 188	1	2240 5' Bam HI Start 251	1
2283 3' Hind III Stop	1	2283 3' Hind III Stop.	1
10x dNTP	5	10x dNTP	5
10x PCR	5	10x PCR	5
H_2O	36.8	H_2O	36.8
Taq	0.2	Taq	0.2
	50		50

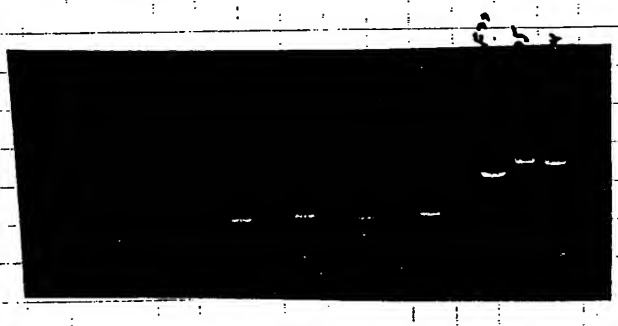
11 tubes ⑦ DNA 1 tube ⑧ control.

HE9MF73/HTPAN08 Protein Exp.

7/26/94

⊖ Control – No DNA.
⊕ Control DNA @ Amplified M13FOR.

PCR Program #00158
95°C 5 min
95°C 60sec
55°C 60 sec
72°C 1 min
} 30X
72°C 7.5 min
Run 5ul on gel.



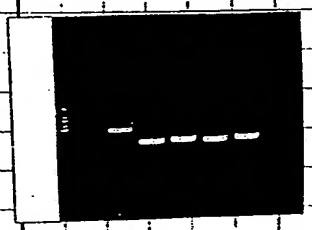
Add Equal Volume 13% PEG/1.6M NaCl
-20°C O/N.

7/27/94

Spin 15 min
1x 70% Wash
Dry pellet
Resuspend in 50ul H_2O.
Run 1ul on gel.

138 HEMF73/H7PAN08 Protein Exp

7/27/94



2-e Look Good
Digest w/ appropriate enzymes

7/28/94

Digest w/ appropriate Enzymes

#(2) A

DNA	10
10X #3	3.0
BamHI	1
H_2O	16
	30

#(2) B

DNA	10
10X #3	3.0
BglII	1
H_2O	16
	30

(3) A

DNA	10
10X 4	3
NCO	1
H_2O	16
	30

(3) B

DNA	10
10X 4	3
Bam	1
H_2O	16
	30

(4) A

DNA	10
10X #4	3
NCO	1
H_2O	16
	30

(4) B

DNA	10
10X #4	3
Bam	1
H_2O	16
	30

HEGMF73/HTPAK08 Protein Exp.

7/28/94

⑤ A	
DNA	10
10X #2	3
Bam	1
H_2O	16
	30

⑤ B	
DNA	10
10X #2	3
H III	1
H_2O	16
	30

⑥ A	
DNA	10
10X #2	3
Bam	1
H_2O	16
	30

⑥ B	
DNA	10
10X #2	3
H III	1
H_2O	16
	30

Incubate 37° C 3 hrs.
Add Alternate Enzyme. Incubate 3hrs.
Reboard

Ligations:

#2	Bam HI / Bgl II	–	pQE 60 & 70. Bam / Bgl II
#3	NCO / Bam	–	pQE60 > NCO/Bam
#4	NCO / Bam	–	pQE60 > NCO/Bam
#5	Bam / H III	–	pDIO > Bam/H III
#6	Bam / H III	–	pDIO > Bam/H III

7/29/94

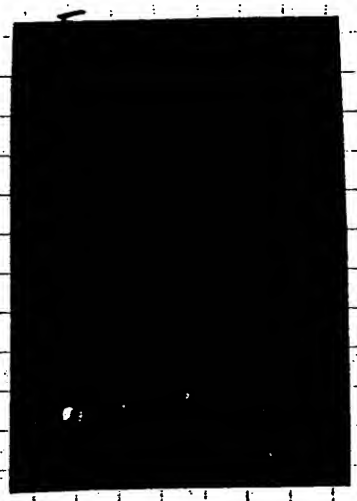
Run on 0.8% LMP gel with 1 Kb ladder.

Cut out of gel –

Gene Clean fragments

140 Protein Express.

7/29/94

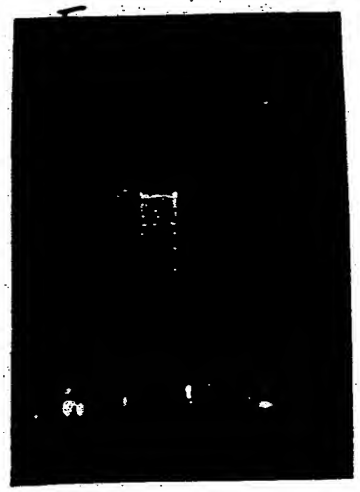


Add 500 µl NaI
Heat 55°C 5min
Mix well
Add 5µl of Glass milk
Let sit RT 2min w/ occasional mixing
Spin 5 sec
Remove Supernatant
Resuspend in 500 µl Wash Buffer
3x [Spin 5sec
Remove Supernatant]
Spin 5sec
Remove all supernatant as possible
Add 30µl of TE
Heat 55°C 5min
Spin 5 sec
Transfer Supernatant to fresh tube
Store -20°C to do ligations

pg 145

Protein Express.

7/29/94



Add 500 µl NaI
Heat 55°C 5 min
Mix well
Add 5 µl of Glass milk
Let sit RT 2 min w/ occasional mixing
Spin 5 sec
Remove Supernatant
3x [Resuspend in 500 µl Wash Buffer.
Spin 5 sec
Remove Supernatant]
Spin 5 sec
Remove all supernatant as possible
Add 30 µl of TE
Heat 55°C 5 min
Spin 5 sec
Transfer Supernatant to fresh tube
Store -20°C to do ligations

pg 145

PROTEIN Expression

pg 145

7/29/97

Set up ligations

DNA	2
Vector	1
10x Buffer	2
H_2O	14
1u DNA ligase	1
	20ul

Store 4°C over Weekend

	DNA fragment		Vector	
1	HEGHFT3505	BamI/BglII	PQE60	BglII/BamI
2	3' BglII Stop		↓	BamI/BglII
3	5' BamI start		PQE70	BglII/BamI
4			↓	BamI/BglII
5		BglII BamI	PQE60	BglII/BamI
6			↓	BamI/BglII
7			PQE70	BglII/BamI
8			↓	BamI/BglII
9	HTPAN0504 5'NCO 25	NCO/Bam	PQE60	NCO/Bam
10	3' BamI Stop	BamI/NCO		NCO/BamI
11	HTPAN0504 5'NCO 185	NCO/BamI		NCO/BamI
12	3' BamI Stop	BamI/NCO	↓	NCO/BamI
13			pQE60	BglII/BamI
14			↓	BamI/BglII
15			PQE70	BglII/BamI
16			↓	BamI/BglII
17			pQE60	BamI NCO/BamI
18				
19	End of DNA fragment.			

8/1/94

Transform M15 cells.

10 µl of ligation
1 µl of 10 ng/µl pBSK
1 µl of 10 ng/µl pD10

Use M15 Chemically Competent cells

DNA or ligation + 100 µl Cells
Incubate on ice 1 hr.
Heat 42°C 45 sec
Put on ice
Add 400 µl LB
Incubate 37°C 1 hr.
Plate 100 µl onto LB + Amp + Kan plates
Incubate 37°C O/N.

8/2/94

pick 12 colonies of 1-8 into 96 Well dish LB + Amp + Kan

pick 24 colonies of 9-12 into 96 Well dish LB + Amp + Kan

PCR #2A+B (1-8)	(100x)		#3 (9+10)	(50x)		#4 (9 11 12)	(50x)
#2245	0.4	#2244	0.4	#2243	0.4		
#2242	0.4	#2241	0.4	#2241	0.4		
10x dNTP	3.0		3.0		3.0		
10x PCR	3.0		3.0		3.0		
Taq	0.2		0.2		0.2		
H2O	23		23		23		
	30 µl		30		30		

Protein Expression

8/2/94

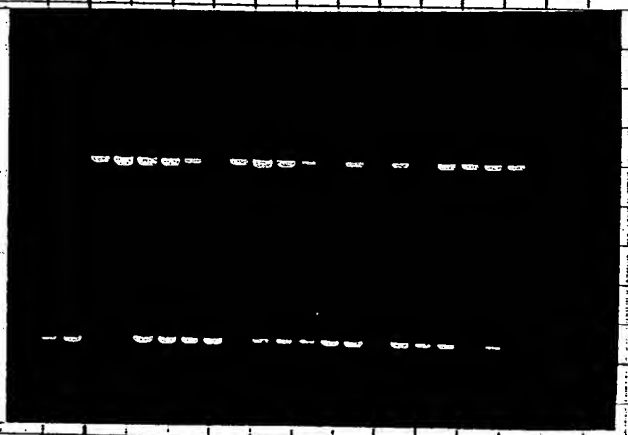
PCR Program #69.

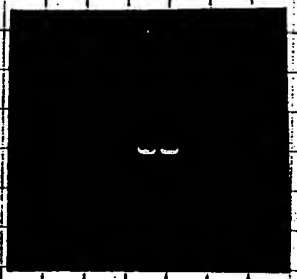
95°C	5min	
95°C	20sec	30x
55°C	20sec	
72°C	1 min	
72°C	7½ min.	

Run 12 µl on 1% TAE gel with 1 Kb Ladder

#2 – No ⊕ – Whole gel Blank –
Re set up ligation again – Make more PCR product (ODH fragment)

#3 –
HTPAD504
5' Nco 251
3' Bam Stop





#4
H+PAD0SS04
5' Nco 185
3' Bam

Protein Expression

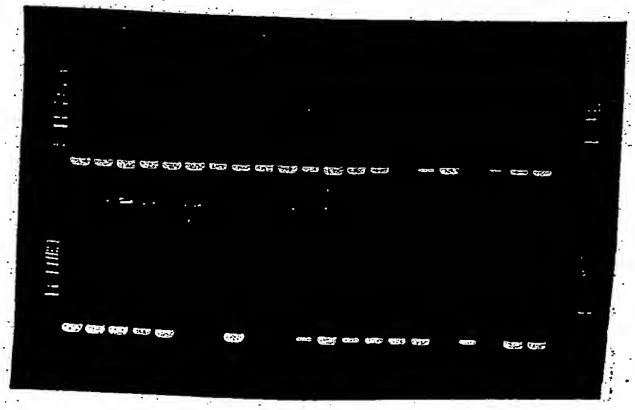
8/12/94

PCR Program #69.

95°C	5min	
95°C	20sec	} 30x
55°C	20sec	} 30x
72°C	1 min	} 30x
72 ~~95~~°C	7½ mn.	

Run 12 ul on 1% TAE gel with 1Kb ladder

#2 - NO ⊕ - Whole gel Blank -
Re set up ligation again - Make more PCR product (DNA fragment)



#3 -
HTPAN504
5' NCO 251
3' Bam Stop

#4
HTPADC85O4
5' Nco 185
3' Bam

148 Protein Expression

8/2/94

Inoculate 3ml LB + Amp + Kan with 1st 4D of each.
into
Incubate 37°C O/N

8/3/94

Add 4ml LB + Amp + Kan
Add 100 mM IPTG to 2mM (140 ul)
Incubate 37°C 5 hrs.
Spin 5 min - 1 ml
Remove supernatant
Resuspend 100 ul H_2O
Add 100 ul 2x Sample Buffer

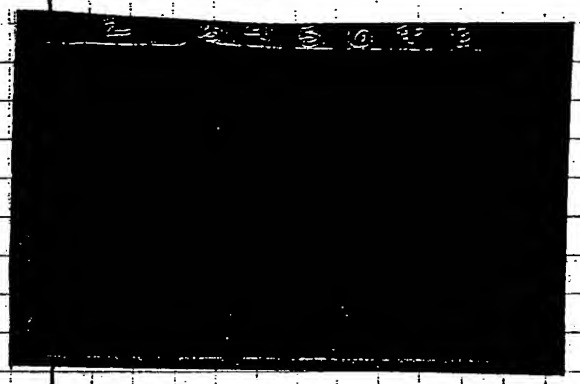
Heat 100°C for 5 min
Chill on ice
Spin 5 min

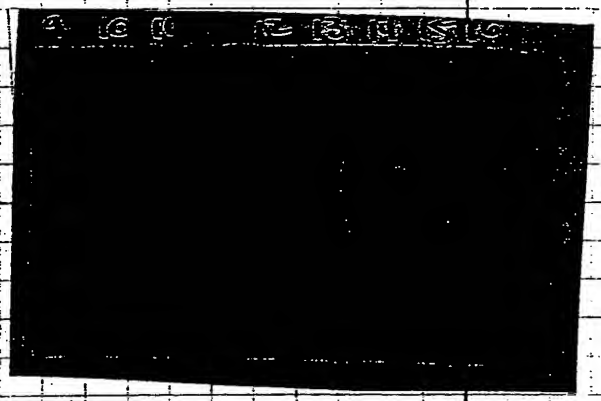
Run 10 ul on 10% Acrylamide gel
with Rainbow Marker
Forgot to have ⊖ control & uninduced
so will re-run. 1 gel with ⊖ controls
and selected samples.

Run 150 V 1 hr
Stain 30 min
DE Stain O/N at RT

8/4/94

HTPANSO4 5'NCO 25! Start. 35' Beam Stop.





HTPANO8SO4 5'NCO 18! Start 3' Beam Stop